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Multiple Unit Correlates of Discrimination Learning in Rats

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MULTIPLE UNIT CORRELATES OF DISCRIMINATION LEARNING IN RATS

by
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for the degree of

Master of Arts

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This thesis submitted by Lynn C. Conrad in partial fulfillment of the requirements for the Degree of Master of Arts from the University of North Dakota is hereby approved by the Faculty Advisory Committee under whom the work has been done.

E. L. Hales
(Chairman)

Dean of the Graduate School

Permission

Title Multiple Unit Correlates of Discrimination Learning in Rats

Department Psychology

Degree Master of Arts

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ABSTRACT

Multiple unit recording is a relatively new electrophysiological recording technique which records spike potentials from many neurons. In several laboratories, systematic changes have been observed during classical and instrumental conditioning but none under other conditioning procedures. The present study attempts to extend the method of multiple unit recording to discrimination learning using rats as experimental animals.

Five permanent electrodes were implanted in each of six rats. The sites implanted were the dorsal medial hypothalamus, the amygdaloid complex, the reticular formation, the medial geniculate, and the inferior colliculus. The animals were subjected to discrimination training which utilized tone as the auditory stimulus and water as the reinforcement.

Neuronal activity from the electrodes was recorded on tape and then transferred to computer cards for statistical analysis. Basically, the number of neuronal spikes for the pre-tone, tone, and post-tone periods were compared in order to judge the effects of reinforcement or non-reinforcement on brain activity.

Generally, there was inhibition of neuronal activity when tones were presented during habituation sessions. However, by the last day

of conditioning, four of the five electrode sites were demonstrating significantly greater neuronal activity after the presentation of a previously reinforced tone. This was not the case upon presentation of a previously non-reinforced tone. Extinction generally resulted in inhibition of activity.

No significant differences were obtained between the reinforced and non-reinforced trials on the last day of conditioning. However, behavioral curves appeared closely associated with neuronal activity.

INTRODUCTION

Multiple unit recording is a relatively new neurophysiological technique which promises to contribute many important findings to the study of the electrical events in the brain. However, since it is new, many basic parameters of the method need to be explored. A limited number of studies have been conducted to this end.

The purpose of this investigation is to apply the method of multiple unit recording to discrimination learning using rats as experimental animals. Basically, there are four questions to be investigated: 1) What effect does an auditory discriminative stimulus have upon neuronal activity? 2) Which sites in the brain react to this stimulus? 3) What relationship is there among pre-stimulus, stimulus, and post-stimulus neuronal activity? 4) Are there any similarities between neuronal activity and behavior? Hopefully, the questions raised in this study will result in further experimentation of multiple unit recording techniques.

CHAPTER I

REVIEW OF THE LITERATURE ON MULTIPLE UNIT RECORDING

Multiple unit recording is a relatively new technique. Essentially, the technique provides a means of sensitively and specifically summarizing activity in the neural tissue surrounding a recording electrode. The technique was initially developed to study nerve fibers which carried an irregular distribution of impulses in response to some stimulus. The study of asynchronous firing of neurons as the result of gustatory (Beidler, 1953, 1957; Pfaffman, 1955) or olfactory (Konishi and Zotterman, 1963) stimuli was thus well suited to this method.

However, in order for the method to be of any use, the asynchronous firing needed to be measured in some way. Thus it was that Beidler (1953) developed an electronic "integrator" that reflected both the frequency and amplitude of the massed unit discharge. Since then, four other laboratories have independently developed similar summarizing devices (Arduini and Pinneo, 1962; Starr and Livingston, 1963; Schlag and Balvin, 1963; and Weber and Buchwald, 1965).

These summarizing devices have resulted in an integrated out-

put which follows rapid changes in both frequency and amplitude of multiple unit activity without being disproportionately influenced by isolated, large amplitude spikes. In contrast, single unit recordings involve measuring the firing pattern of just one neuron. Therefore, the small sample of neuronal activity necessitated by such unit recording, combined with the great variability in unit activity patterns, have made generalizations difficult when using single unit recording techniques.

The name "multiple unit activity" has been chosen because it appears that the macroelectrode employed yields a record of the action potentials of an undetermined number of neurons. These neurons are spontaneously firing (called ongoing or background activity) or are induced to fire by some stimulus (Schlag and Balvin, 1963; Buchwald, Halas, and Schramm, 1965a; Winters et al., 1967; Halas, Beardsley, and Sandlie, 1969; Halas and Beardsley, 1969, 1970a, 1970b). Halas and Beardsley (1968) reported that the multiple unit method records rapid voltage changes from spikes near the tip of the electrode ranging from a distance of 0.5 to 1.0 mm. At the present time, it cannot be determined exactly what contributes to an overall increase or decrease in activity. Possibly, the same units may fire more rapidly during a stimulation period, or perhaps other previously silent units may begin to fire.

Until just recently, the multiple unit activity and integrator line were traced on an oscilloscope, photographed by a high speed

movie camera, and the film was displayed for interpretation. It was possible to count the distinct spikes that occurred, but such a count for any amount of data was extremely tedious and the judgments of fluctuations in the integrator line were subjective. However, a method of frequency and amplitude analysis of multiple unit activity with digital output (Halas, Kalbfleisch, Olson, and Walker, 1971) has been developed which eliminates the need for an integrator device. Neuronal activity received from various macroelectrodes is removed simultaneously on a seven track tape recorder-reproducer. The data output is then punched onto computer cards, and is easily available for statistical analysis and interpretation.

The studies in the United States utilizing multiple unit recording have taken two basic foci: ongoing activity (background activity, massed unit activity) itself; and the neuronal firings as a response to some explicit training procedure. The former type typically use long and complex stimuli such as the observation of responses to white noise or anaesthesia or sleep over several minutes or hours. The latter type study neuronal responses which occur in relation to some explicit training procedure (e.g., Pavlovian classical conditioning or instrumental conditioning).

With respect to the first type of study, Winters (et al., 1967) determined that reticular activity is greater during rhombencephalic sleep than during the awake state or slow wave sleep. These results are consistent with the findings of Schlag and Balvin (1963) who re-

corded EEG and multiple units simultaneously. They showed that when large alpha waves were preceded and accompanied by increased multiple unit activity, the neuronal activity seemed to be actively inhibiting the EEG activity. Also, in agreement with Winters et al., they found a negative linear relationship between the degree of multiple unit activity and the amplitude of the EEG waves under the conditions of paradoxical sleep (multiple unit activity greatest), alertness (multiple unit activity moderate), and deep sleep (multiple unit activity least).

Podvoll and Goodman (1967) observed that multiple unit recordings vary consistently with observed behavior even under conditions when the EEG appeared unrelated to behavior. Starr and Livingston (1963), when investigating sites in the inferior colliculus and medial geniculate, determined that the level of spontaneous activity recorded from all the sites tended to rise in association with bodily movements, the rise being greatest at the inferior colliculus. However, under anaesthesia, the medial geniculate did demonstrate a large, sustained response, thereby suggesting that perhaps, in the waking state, the medial geniculate is "protected" from response to steady sound by some mechanism which is obliterated by anaesthesia. Goodman and Mann (1967) and Podvoll and Goodman (1967) also reported detectable differences in multiple unit activity under different depths of anaesthesia.

Several laboratories have explored the relationship between EEG

and multiple units by utilizing the same recording electrode simultaneously (Schlag and Balvin, 1963; Buchwald, Halas, and Schramm, 1965a, 1966b; Winters, 1967). Buchwald et al., found that the most common relationship between neuronal spike populations and EEG wave activity, both at cortical and subcortical levels, is one of independence. They regard multiple unit recording as being a much more sensitive index of changing neuronal activity than the EEG. As previously cited, Schlag and Balvin (1963) regard location of electrodes and the degree of alertness of the animal as important parameters to consider when judging the relationship between multiple units and the EEG. Winters (et. al., 1967) also demonstrated independence between EEG and multiple unit recordings.

A number of studies have been conducted concerning the relationship between neuronal firings and auditory and visual stimuli (Starr and Livingston, 1963; Galin, 1964; Podvoll and Goodman, 1967; Halas and Beardsley, 1968). Galin found much variability to tones and white noise in the inferior colliculus (IC). White noise in the IC usually increased unit activity whereas tones (500 Hz) generally inhibited them. Starr and Livingston reported increasing neuronal responsiveness to white noise as they recorded from higher to lower CNS centers. According to Podvoll and Goodman, multiple unit responses to white noise habituated at thalamic levels (medial geniculate, centrum medianum) but not below in the auditory system. Halas and Beardsley, engaged in further research in this area, showed that

responses to a 1500 Hz tone occurred in the inferior colliculus, an auditory nucleus, but did not occur to light. Conversely, they demonstrated that light, but not tone, caused responses in the superior colliculus, a visual nucleus.

Another application of the multiple unit technique has been the recording of neuronal firing changes which occur as a result of behavioral conditioning (Buchwald, Halas, and Schramm, 1965b, 1966a, 1966b; Halas and Beardsley, 1969, 1970a, 1970b; Halas, Beardsley, and Sandlie, 1969). Regular and systematic changes (Buchwald, Halas, and Schramm, 1965b) occurred in multiple unit activity in the primary projection system of the conditioned stimulus (tone) and the non-specific reticular system during the development of a classically conditioned response. There were no observable changes in the unconditioned stimulus pathway. In addition, a decrease in multiple unit activity was observed on non-reinforced trials with only tone presented.

Halas and Beardsley (1969, 1970a, 1970b) extended their research to include both classical and instrumental conditioning. In their earliest paper (1969), they reported that different neuronal responses appeared in the cochlear nucleus of a cat during classical and instrumental conditioning. During classical aversive conditioning, the conditioned neuronal response was a sustained increase in neuronal activity which remained relatively unchanged throughout acquisition. However, during instrumental avoidance conditioning,

there were three different patterns of neuronal responses: a sustained increase similar to the response observed during classical conditioning followed by a second pattern consisting of a brief onset, and a final pattern consisting of an initial positive onset followed by a marked decrease in neuronal activity. This research and a later study (Halas and Beardsley, 1970b) suggest that the early phases of instrumental avoidance conditioning involve classical conditioning, and that the differentiation of classical and instrumental conditioning occurs quite low in the brain stem. These same changes were observed when recording from the inferior colliculus of cats during classical and instrumental conditioning (Halas and Beardsley, 1970a).

Halas, Beardsley, and Sandlie (1967) attempted to analyze the neuronal responses during classical and instrumental conditioning and to determine whether some structures show conditioning earlier than others and in what sequence. Using an auditory stimulus, they obtained results which indicated that conditioning occurred first in the reticular formation and progressed upward from the cochlear nucleus to the auditory cortex.

Beardsley (1968) was unsuccessful in his attempt to study discrimination learning. However, some studies have been done in this area utilizing single unit recording techniques (Phillips and Olds, 1969; Travis and Sparks, 1967, 1968; Travis, Hooten, and Sparks, 1967). The Phillips and Olds (1969) study used three tones which accompanied either food or water or no reinforcement. For a hungry

animal, sustained or increased firing rates were elicited by a "food" tone and lowered rates by the other tones. Comparable results were obtained with thirsty animals and a "water" tone. Due to a two second waiting period between the onset of a tone and reinforcement, the neuronal firings were based primarily on the organism's anticipatory state rather than overt behavior. Therefore, it may be suggested that single cells in the midbrain, the area sampled, can make discriminatory responses to different sensory signals. Similarly, discriminative learning has been accompanied by accelerated firing rates in the reticular formation (Sparks and Travis, 1968) and the globus pallidus (Travis, Hooten, and Sparks, 1967).

The present study was designed to expand the multiple unit recording literature to include discrimination learning data. In addition, rats were chosen as experimental animals in order to determine their adequacy in such types of experimentation.

CHAPTER II

METHOD AND PROCEDURE

Six adult Sprauge-Dawley rats were implanted with five permanent electrodes. Sites were chosen in the primary auditory system (inferior colliculus, IC; medial geniculate, GM); the amygdaloid complex, AC; the reticular formation, RF; and the dorsal medial hypothalamus, DMH. The atlas of Pellegrino and Cushman (1967) was used to determine the placement of the electrodes. Appendix A reports the Pellegrino-Cushman coordinates.

At the beginning of surgery each rat was given an appropriate dosage (.10 cc per 100 gram weight) of sodium pentobarbital (3.25% solution) to render him unconscious. After placement in a Kopf stereotaxic instrument, a longitudinal incision was made on the scalp and the cranium cleaned of muscle and connective tissue. The appropriate coordinates for electrode placement were marked on the skull, and nine holes were drilled through the skull for the five electrode placements and four holding screws.

Using an electrode carrier, each electrode was lowered slowly into the brain. As the electrode passed through various nuclei, changes in multiple unit activity were quite obvious. The multiple

unit activity was monitored visually on the oscilloscope as well as audibly on a loudspeaker system. After each electrode reached its designated site, it was cemented to the skull with dental cement.

Stainless steel screws were screwed into the skull to provide stability for the head cap and also for use as an indifferent lead. The electrodes were soldered to an Amphenol plug and dental cement was used to adhere the plug to the skull. A few stitches were taken in both ends of the incision to draw the skin around the plug. After penicillin was administered to prevent infection, each rat was given 5-7 days to recover from surgery.

The electrodes were made of size 00, stainless steel insect pins which were insulated with Epoxylite. About 0.1-0.3 mm of the electrode tip was bared.

Neuronal activity was recorded simultaneously from the five electrodes following the procedure described in Halas et al., 1971. The neuronal inputs were amplified by Tektronix 122 preamplifiers and fed through modified high pass filters. These amplified filtered signals were then passed to a seven-track Minnesota Mining and Manufacturing Company C100 analog tape recorder-reproducer. For observation purposes during recording, the recorded signal of one track was monitored simultaneously from the reproduce heads onto a Tektronix dual beam 565 oscilloscope.

For frequency analysis of the data, the tape was played back one track at a time. The tape recorder-reproducer output was con-

nected to the vertical input of the Tektronix 565 oscilloscope which in turn sent a signal to the digital analyzer. In the digital analyzer, a digital logical pulse was generated for each neuronal spike which exceeded a certain minimum voltage level (threshold). The data output of the system was automatically punched on computer cards by means of an IBM 024 card punch connected to the digital analyzer.

Training Conditions

The six rats were exposed to sixteen days of training. Each animal was individually trained in a modified operant conditioning box in a sound-deadened room. The box contained a Leheigh-Valley Electronics 114-02 water dipper which presented a water reinforcement. The speaker for the tone was mounted one meter directly in front and above the mounted water dipper.

The animals were deprived of water for 23½ hours before each training session. Twenty trials were given to each animal on each of the sixteen days. The trials were presented at random intervals, the mean inter-trial interval being 35 seconds $\pm$ 20 seconds.

Water Habituation

The first three days consisted of habituation to the water dipper. The dipper was presented for six seconds each trial. By the third day, all the animals except one readily drank when water was available. The animal which didn't drink was eventually dropped from the study because he huddled in a corner of the operant chamber

for the full sixteen days of training.

Tone Habituation

The next five days were devoted to tone habituation. Ten high tones (1500 Hz) and ten low tones (500 Hz) were presented in random permutations to each animal every day. The water dipper was not presented.

Conditioning

Three animals were chosen to receive water reinforcement upon hearing the high tone, and three animals received reinforcement upon hearing the low tone.

Again, each animal received twenty trials per day including ten trials of high tones and ten trials of low tones. However, during these five days of conditioning, each animal was only reinforced with water during the ten trials involving the appropriate high or low tone.

Extinction

The last three days of training involved extinction. Each animal received the ten high and ten low tones per day, but no tones were reinforced. By the third day of extinction, no animals were attempting to find the water dipper and the study was terminated.

Recording Conditions

Each of the twenty trials per day per animal involved 12 seconds of recording time. A pre-tone period lasted for four seconds during which the animal's basal level of neuronal activity was recorded.

Then, either a high or low tone was presented for six seconds, the water dipper being presented one second after the start of the tone. During the two second post-tone period, neither the tone nor water was present. All neuronal spikes exceeding a predetermined threshold during the pre-tone, tone, and post-tone periods were recorded on computer cards as previously explained.

Behavioral measures were also taken. The number of licks each animal made on the water dipper was recorded for each trial using a contact relay.

Histological verification of the electrode placements was not attempted.

CHAPTER III

RESULTS

A sample of the total data collected was used for analysis purposes. The days sampled were Tone Habituation 5; Conditioning 1, 3, and 5; and Extinction 1 and 2. For each of these days, four of the twenty trials were chosen; namely, trials 5, 10, 15, and 20. On those days where an even number of high and low tones did not occur on the selected trials, the nearest trials which filled the requirement of two high and two low tones were chosen. On the selected days and trials, data was collected for each animal's five electrodes.

The average number of neuronal spikes exceeding the predetermined threshold were obtained for the pre-tone, tone, and post-tone periods. In addition, the number of neuronal spikes occurring in the one second interval between the onset of tone and presentation of the water dipper was determined. This information was then analyzed according to the four basic questions presented in the introduction of this paper. These questions will be now dealt with in turn.

Effect of an auditory discriminative stimulus upon neuronal activity

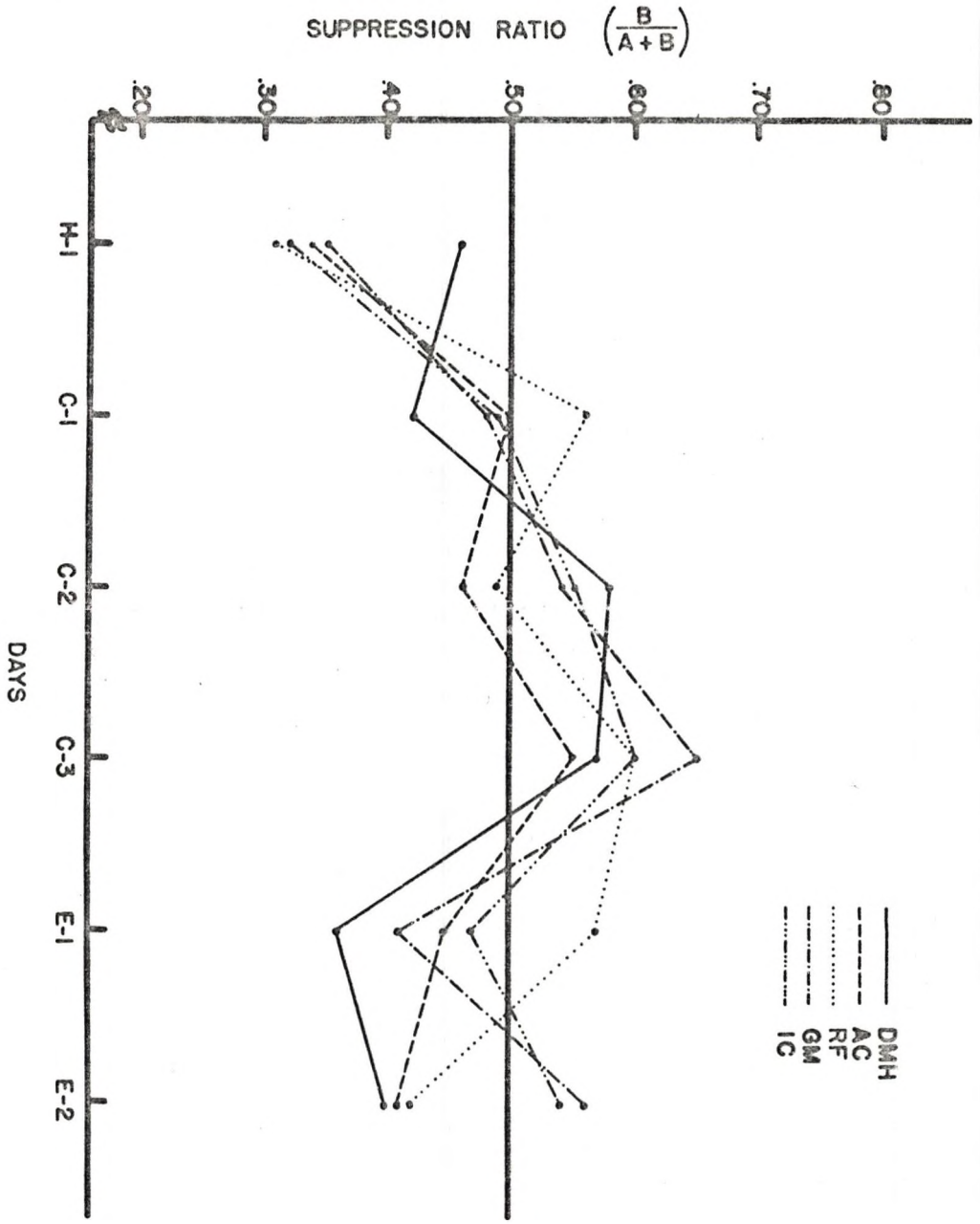
Of particular importance to this experiment is the study of the neuronal activity occurring during the first second of tone. At this

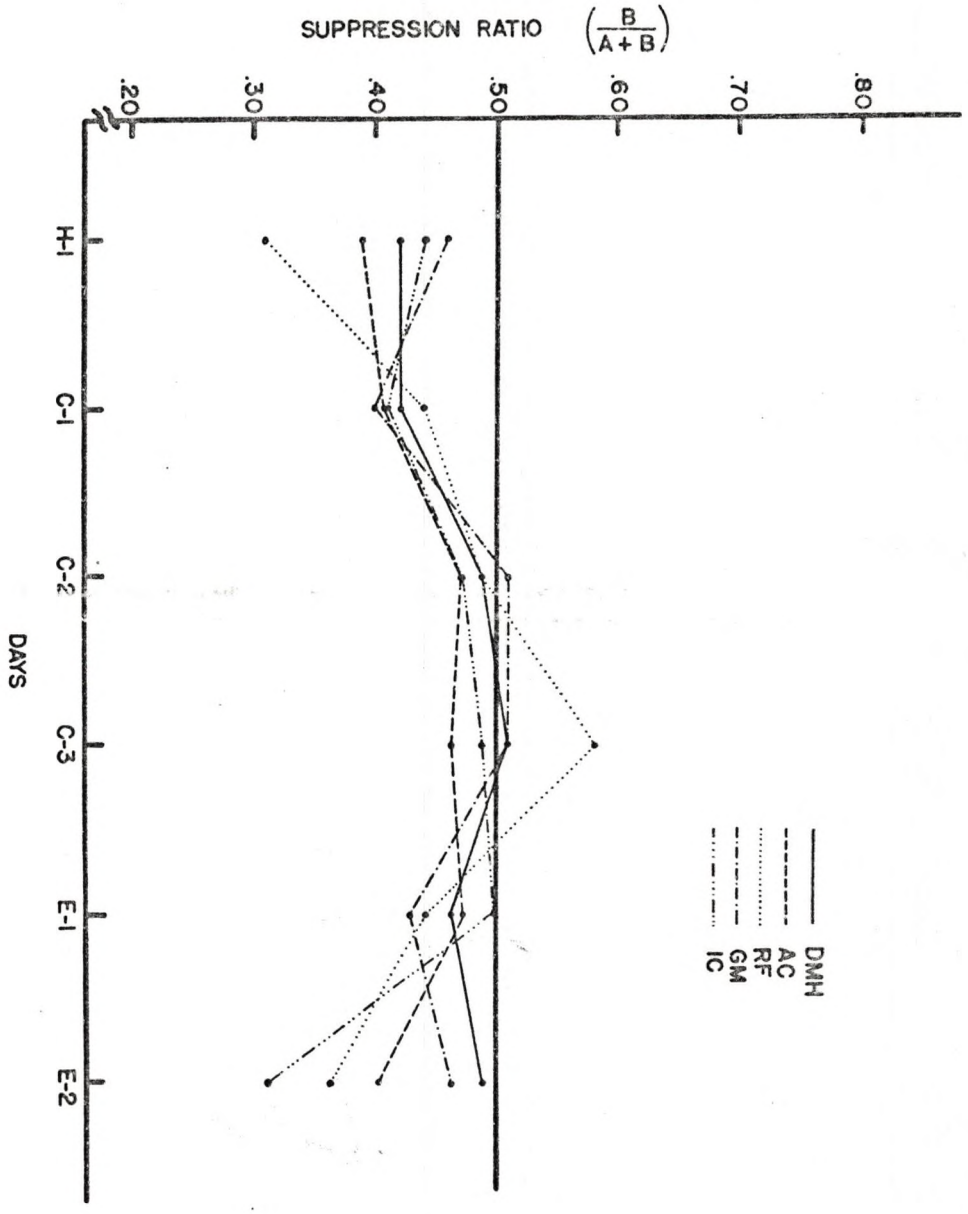
point, the animal is in an anticipatory state and the effect of the tone itself can be analyzed without the motor interference of consumatory responses.

To study this anticipatory period, the level of activity of the pre-tone period was compared to the activity occurring in the one-second interval following the presentation of a tone. As previously noted, a given tone was consistently followed either by reinforcement or no reinforcement. Annau and Kamin's (1961) measure of response suppression (cf. Campbell and Church, 1969) was used as a measure in attempting to study the anticipatory period because this method involves a measure of performance prior to treatment and performance during treatment. In the present study, the pre-tone period represents (A) and the one-second interval after the start of the tone represents (B). The basic formula used is B/(A+B). A subject responding at the same rate during the (A) and (B) periods would have a ratio of .50. If he responded more during period (B), his ratio would rise, and if he responded less during period (B), his ratio would go down.

The data for all rats averaged together is presented in Figure 1 and Figure 2. Figure 1 consists of suppression ratios calculated for the differences between periods (A) and (B) when the tone presented is one which is reinforced. Figure 2 presents the measure when the tone is one which is not reinforced.

As will be noted in Figure 1, on the day of Tone Habituation


SUPPRESSION RATIO $\left(\frac{B}{A+B}\right)$
.20
.30
.40
.50
.60
.70
.80
H-1
C-1
C-2
C-3
E-1
E-2
DAYS
DMH
AC
RF
GM
IC


SUPPRESSION RATIO $\left(\frac{B}{A+B}\right)$
.20
.30
.40
.50
.60
.70
.80
H-1
C-1
C-2
C-3
E-1
E-2
DAYS
DMH
AC
RF
GM
IC

(H-1), there was marked inhibition of neuronal activity in all five electrodes during the one-second tone period. However, by the last day of conditioning (C-3), all sites were exhibiting more activity during the one-second period than during the pre-tone period. Generally, extinction resulted in inhibition during the one-second presentation.

Figure 2 exhibits more horizontal curves than does Figure 1. Again, during Tone Habituation, there is an inhibition of neuronal activity during the one-second tone period. There is a slight rise in activity over days of conditioning, and extinction seemed to create some inhibition during the tone period.

Differences in neuronal activity among electrodes

Although the general trends of the five electrodes in Figure 1 and Figure 2 seem to be similar, there are some differences due to electrode site. Sandler's A-statistic (Sandler, 1955) was used to determine if there were any significant differences in neuronal activity between days H-1 and C-3 for each electrode.

Table 1 presents the results of the differences between days H-1 and C-3 when the tone was followed by reinforcement. The amygdaloid complex, the medial geniculate, and the inferior colliculus all acted significantly (.01 level) different on the last day of conditioning. The reticular formation was significantly different at the .05 level. Only the dorsal medial hypothalamus failed to demonstrate any increased level of activity by the final day of conditioning.

When Sandler's A-statistic was applied to days H-1 and C-3 of the non-reinforced tones, only one difference was significant, the reticular formation. All other sites exhibited the same general level of activity on both the habituation and final conditioning days. These results are given in Table 2.

TABLE 1

VALUES OF A FOR THE DIFFERENCE OF NEURONAL ACTIVITY BETWEEN DAYS H-1 AND C-3 ON REINFORCED TRIALS[a]

Electrode	Values of A	Significance
DMH	3.356	N.S.
AC	.197	$p < .01$
RF	.244	N.S.
GM	.178	$p < .01$
IC	.190	$p < .01$

[a]df = 9. All probabilities are at the .01 level, one-tailed test.

TABLE 2

VALUES OF A FOR THE DIFFERENCE OF NEURONAL ACTIVITY BETWEEN DAYS H-1 AND C-3 ON NON-REINFORCED TRIALS[a]

Electrode	Values of A	Significance
DMH	5.485	N.S.
AC	.973	N.S.
RF	.177	$p < .01$
GM	.682	N.S.
IC	1.445	N.S.

[a]df = 9. All probabilities are at the .01 level, one-tailed test.

Analyses were then done to determine if the level of activity was significantly different within electrodes upon hearing a reinforced or non-reinforced tone on a particular day. The last day of conditioning, C-3, was chosen. Unfortunately, no electrode showed a significant difference. These results are presented in Table 3.

TABLE 3

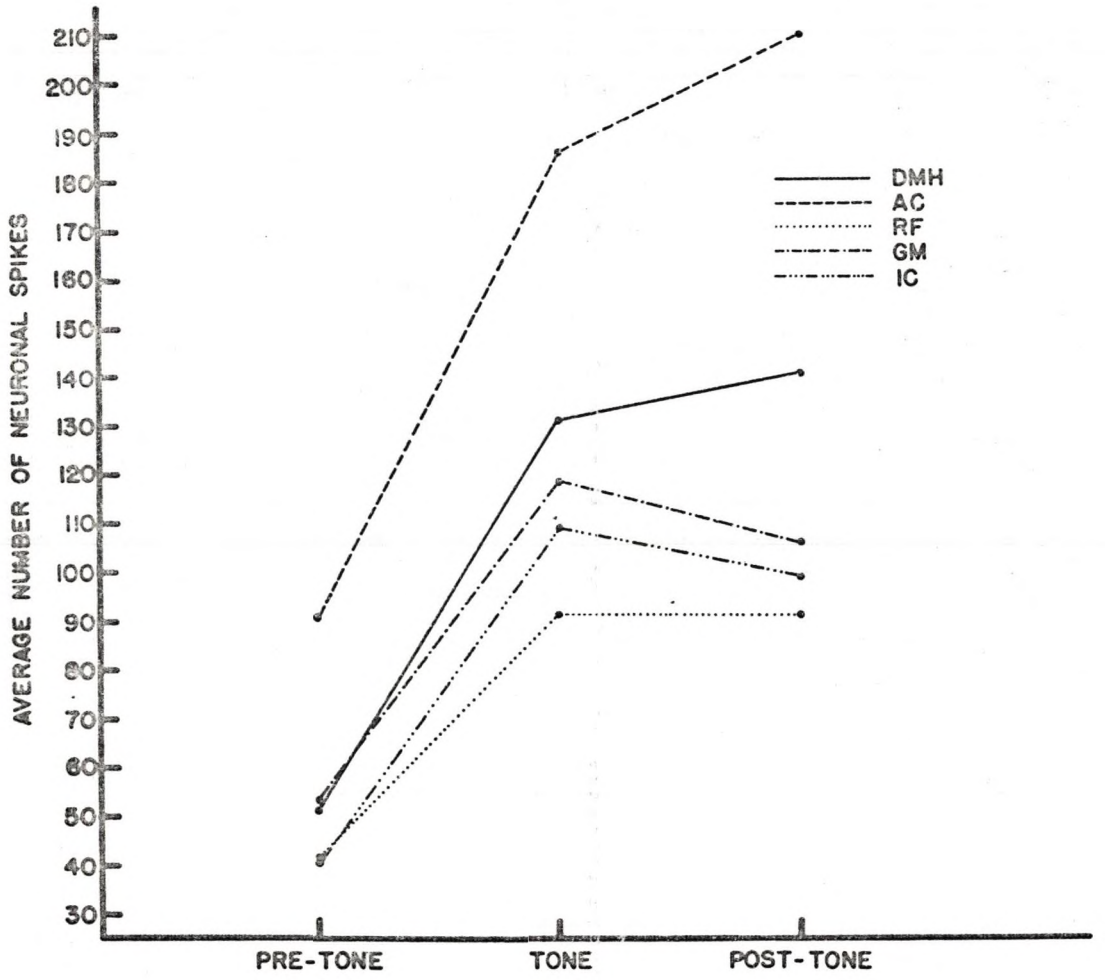
VALUES OF A FOR THE DIFFERENCE OF NEURONAL ACTIVITY WITHIN ELECTRODES UPON PRESENTATION OF A REINFORCED OR NON-REINFORCED TONE ON DAY C-3[a]

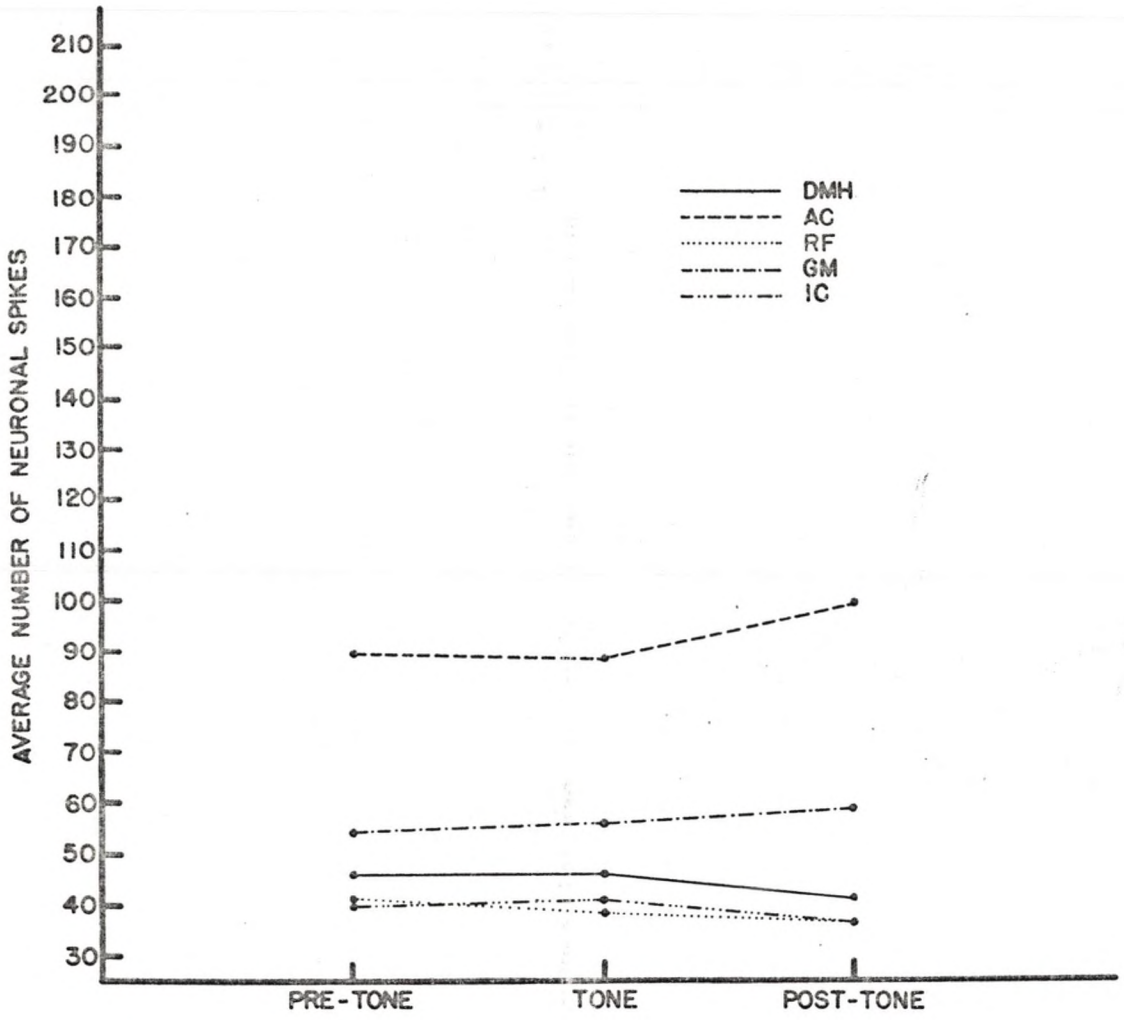
Electrode	Values of A	Significance
DMH	2.954	N.S
AC	1.083	N.S
RF	27.064	N.S
GM	2.318	N.S
IC	.791	N.S

[a]df = 4. All probabilities are at the .01 level, one-tailed test.

Relationship of pre-tone, tone, post-tone periods

When the total 12 second recording period was examined, certain interesting findings appeared. Figure 3 presents the data of different electrode activity during the pre-tone, tone, and post-tone periods when the tone was followed by reinforcement. The tone period includes the one-second interval following presentation of the tone and the five-second interval in which both the tone and water dipper are present. Figure 4 presents the activity data when the tone was not reinforced. For both these figures, all animals and all trial days were combined. First, it may be noted in both figures that the basal level of pre-tone activity differs among electrodes, the activity being greatest in the amygdaloid complex. Secondly, in Figure 3, all


AVERAGE NUMBER OF NEURONAL SPIKES
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
PRE-TONE
TONE
POST-TONE
DMH
AC
RF
GM
IC


AVERAGE NUMBER OF NEURONAL SPIKES
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
PRE-TONE
TONE
POST-TONE
DMH
AC
RF
GM
IC

five electrodes showed an increase of neuronal activity of comparable magnitude during the six-second tone period. In Figure 4, there were no increases in activity during the tone period. Finally, Figure 3 demonstrates that the high level of activity exhibited during the tone period was maintained throughout the post-tone period when the animal was no longer receiving reinforcement. There were no observable differences (Figure 4) among the three periods when the tone was not reinforced.

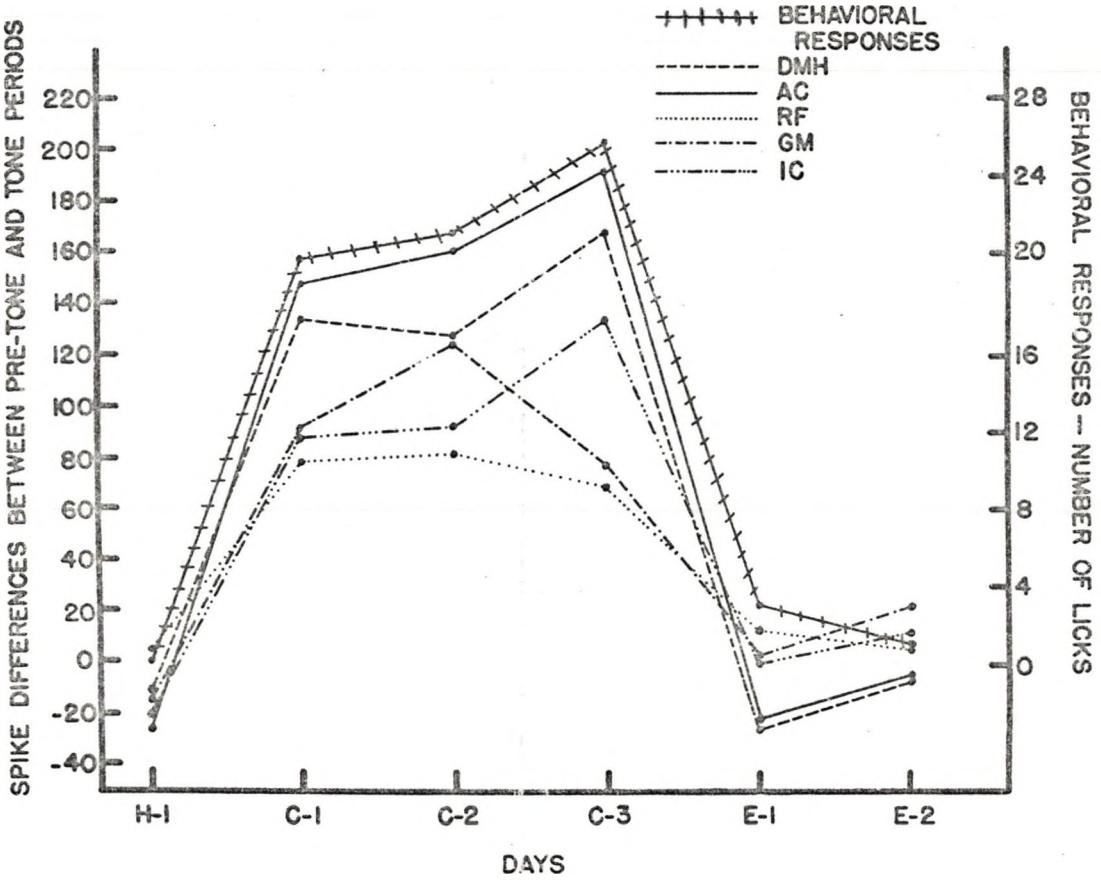
Similarities between neuronal activity and behavioral responses

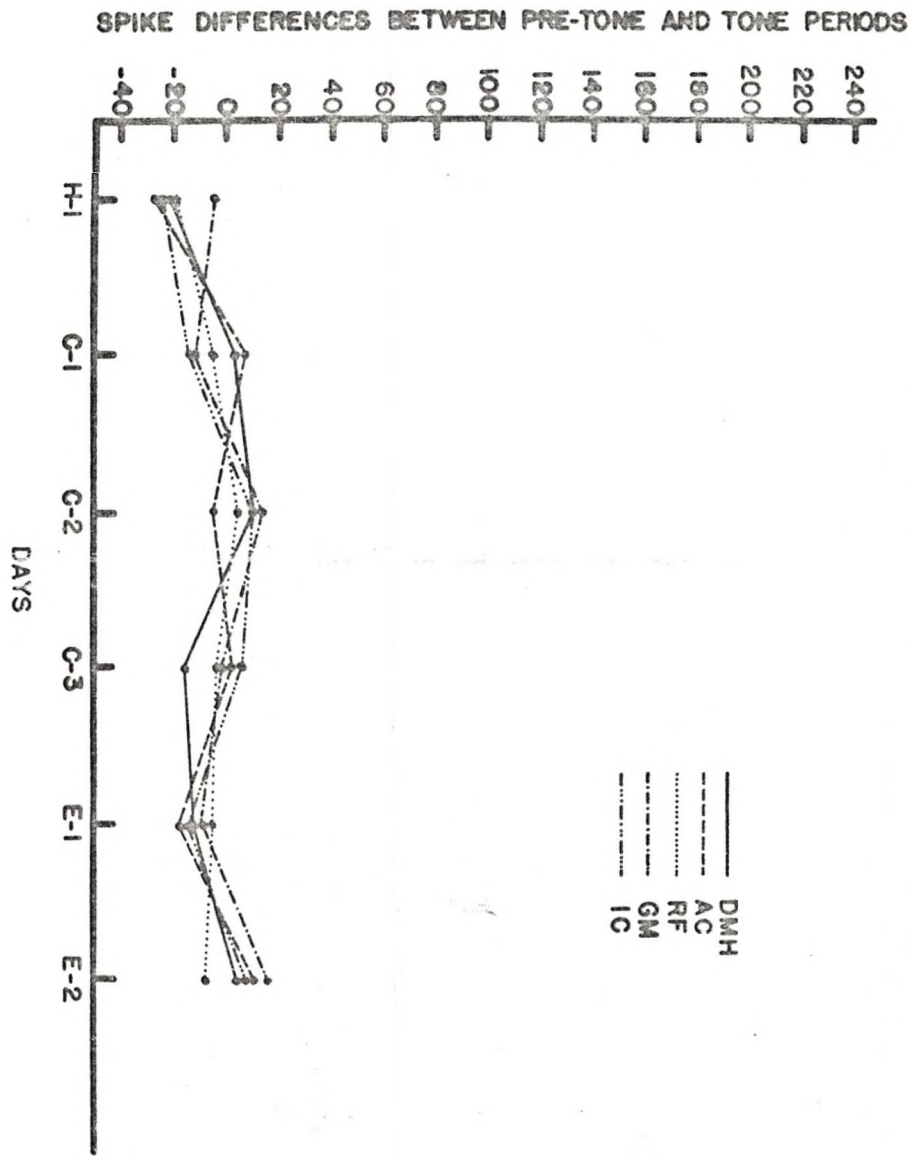
In order to respond to the question of the correlation between neuronal activity and behavioral responses, the six-second tone period was again treated as one unit. In this analysis, however, the absolute magnitude of spike differences between the pre-tone and tone periods was used. Figure 5 presents the data for the reinforced tones, and it also presents the behavioral data; specifically, the number of licks on the water dipper during the five-seconds when water reinforcement was available.

Again, the inhibition of activity during the tone period is evident on the day of tone habituation, H-1. The number of behavioral responses is a negatively accelerating function over days of conditioning. Interestingly enough, the dorsal medial hypothalamus, the amygdaloid complex, and the inferior colliculus all produced curves which are essentially parallel to the behavioral curve. Neither the reticular formation nor the medial geniculate demonstrated

these negatively accelerating curves.

Figure 6 presents the data of the non-reinforced tones for comparison's sake, although the behavioral data was eliminated because the water dipper was not presented and consequently there was no licking response. In general, there was little increased neuronal activity during conditioning days, and the inhibition effect was present during habituation and extinction.


BEHAVIORAL RESPONSES
DMH
AC
RF
GM
IC
SPIKE DIFFERENCES BETWEEN PRE-TONE AND TONE PERIODS
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
BEHAVIORAL RESPONSES -- NUMBER OF LICKS
28
24
20
16
12
8
4
0
H-1
C-1
C-2
C-3
E-1
E-2
DAYS


SPIKE DIFFERENCES BETWEEN PRE-TONE AND TONE PERIODS
-40
-20
0
20
40
60
80
100
120
140
160
180
200
220
240
H-1
C-1
C-2
C-3
E-1
E-2
DAYS
DMH
AC
RF
GM
IC

CHAPTER IV

DISCUSSION

Although this study was intended for exploratory purposes, a certain number of positive results were found. It was determined that rats can be suitable laboratory animals for multiple unit recording techniques. Very careful surgical procedures must be followed, however, for the mortality rate of the rats tended to be quite high (60%). It is hoped that in the future more use of rats will be made in this area for they have definite advantages of small size, little expense, and uncomplicated surgical procedure.

Effect of an auditory discriminative stimulus upon neuronal activity

As was noted in Figure 1 and Figure 2, there were differences in neuronal activity following discrimination training. On the day of tone habituation, all electrode sites indicated an inhibition of activity upon presentation of a high or low tone. This observation is consistent with the findings of Galin (1964) and Buchwald, Halas, and Schramm (1965a). Galin dealt specifically with the inferior colliculus while Buchwald et al., demonstrated such an effect in the medial geniculate.

However, by the last day of conditioning, no electrode demon-

strated inhibition of activity upon presentation of a reinforced tone (Figure 1). Also, in Figure 2, there was no inhibition of neuronal activity by day C-3 when tones were not reinforced. One would expect the level of neuronal activity presented in Figure 1 to be increased to some extent, and the level of activity presented in Figure 2 to be markedly lower if the neuronal activity can be assumed to parallel overt behavioral data. However, it is possible that the amount of inhibition generated by a non-reinforced tone is reduced due to concurrent reinforced tones; and, conversely, the activity resulting from the presentation of a reinforced tone is reduced due to the inhibition effect of non-reinforced tones. Therefore, the lack of significant differences between reinforced and non-reinforced trials may be due to interacting gradients.

During extinction there was again marked inhibition of activity in most sites upon presentation of previously reinforced or non-reinforced tones. It is interesting, however, that the two sites which did not show inhibition (Figure 1) during extinction of reinforced tones were the medial geniculate and inferior colliculus, both specific regions of the main auditory pathway. Yet these sites did demonstrate inhibition of activity during extinction upon presentation of a previously non-reinforced tone. From these facts, it possibly can be assumed that the auditory pathway is capable of discrimination learning to some undetermined extent.

Differences in neuronal activity among electrodes

No significant results were obtained between the reinforced and

non-reinforced electrodes on the last day of conditioning. Therefore, in order to assess how each electrode compared to itself over time, days H-1 and C-3 were compared for each electrode, day H-1 being a base-line for subsequent activity. With this analysis, the amygdaloid complex, the medial geniculate, the inferior colliculus, and the reticular formation demonstrated significantly more activity on the last day of conditioning. Since this data is anticipatory neuronal activity data rather than consumatory behavioral data, it is logical that the dorsal medial hypothalamus, regarded as important as food consumption, is not significant. Also, since the stimulus is auditory, it is important that the medial geniculate and the inferior colliculus did demonstrate significant increased levels of activation.

The amygdaloid complex, as part of the limbic system, is involved with motivational and emotional aspects of behavior. Therefore, it could be assumed that the animal was discriminating between reinforced and non-reinforced tones, and his increased activity upon hearing a reinforced tone was due to the motivational aspects of receiving a water reinforcement.

Only the reticular formation showed a significant difference between day H-1 and C-3 with presentation of both reinforced and non-reinforced tones. Sparks and Travis (1968) determined that discriminative learning caused accelerated firing rates in the reticular formation upon the presentation of a positive discriminative stimulus. Perhaps our finding of significantly greater activity on day C-3 for

both reinforced and non-reinforced tones is due to the reticular formation's role in level of arousal. If this is the case, the level of arousal is increased upon the presentation of any tone, reinforced or non-reinforced, if the animal has been exposed to discrimination training.

Relationship of pre-tone, tone, and post-tone periods

When considering the data obtained from the total twelve second recording period, it must be stressed that this data includes level of activity during the actual licking response of the animal. This data was examined in order to assess the statements of Podvoll and Goodman (1967) and Starr and Livingston (1963), who observed that the level of specific neuronal activity tended to rise in association with bodily movements.

Similar results indicated that when the animal was presented water reinforcement, all five sites demonstrated greatly increased activity during the stimulus period (Figure 3). However, when no licking response was possible (Figure 4), there was no difference between the pre-stimulus and stimulus periods.

However, the post-stimulus period was also considered, it will be noted in Figure 3 that generally the high levels of response during the stimulus period were maintained throughout the post-stimulus period. The medial geniculate and the inferior colliculus, auditory nuclei, decreased in activity somewhat when the tone was discontinued. The amygdaloid complex, a motivational center, increased its activity

greatly during post-stimulus conditions, although no bodily movements associated with licking were possible. The same was true of the dorsal medial hypothalamus.

Similarities between neuronal activity and behavioral responses

The data of Figure 5 and Figure 6 is essentially the same as that discussed just previously, except that in this case the electrodes had been presented individually over days of training. The post-stimulus period data was eliminated, for it was essentially the same as the stimulus period data.

Again, the statements of Podvoll and Goodman (1967) and Starr and Livingston (1963) are supported in that the levels of activity increased in every electrode site when licking was possible (Figure 5). No such increases were observed when licking was not present (Figure 6).

Three of the electrode sites almost directly parallel the behavioral curve, these being the dorsal medial hypothalamus, the amygdaloid complex, and the inferior colliculus. Although it is not possible to explain this occurrence with any definitive statement, it is interesting that the medial geniculate data failed to parallel the inferior colliculus curve since they are both nuclei of the main auditory pathway.

Certain recommendations can be made to facilitate further work in this area of multiple unit recording. First, it is recommended that the total training period be lengthened, including more habit-

uation, conditioning, and extinction days. This is important for it cannot be determined from the present study if asymptotic levels were obtained. Judging from the slopes of the obtained curves, it is doubted that these levels were reached.

Secondly, and most importantly, it is necessary to lengthen the anticipatory period which extends from the moment of presentation of the tone until the appearance of the positive reinforcement. The one-second anticipatory period was simply not adequate to present reliable averages of neuronal spike activity.

APPENDIX

TABLE 4

PELLEGRINO-CUSHMAN COORDINATES FOR EACH SITE IMPLANTED

Site	Anterior	Lateral	Height
Dorsal Medial Hypothalamus (Right)	(-0.4)	(0.5)	(9.0)
Amygdaloid Complex (Left)	(-0.8)	(4.5)	(9.5)
Medial Geniculate (Right)	(-3.4)	(3.0)	(7.0)
Reticular Formation (Left)	(-5.6)	(2.0)	(7.0)
Inferior Colliculus (Right)	(-7.2)	(2.0)	(3.5)

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